Does Vancomycin Resistance Affect Outcome in Patients With *Enterococcus faecium* Bacteremia?

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YALE UNIVERSITY

2003

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A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by
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YSM 2003

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The impact of vancomycin resistance on the outcome of patients with *E. faecium* bacteremia has not been definitively established, but it is an important factor in allocating hospital resources for controlling antibiotic-resistant organisms. This study is a retrospective analysis comparing the outcome of all patients who developed both vancomycin-sensitive (VS) and vancomycin-resistant (VR) *E. faecium* bacteremia at Yale-New Haven Hospital between 1992 and 2000. All patients with *E. faecium* bacteremia were evaluated with respect to risk factors for development of VR *E. faecium* bacteremia. Cases (patients with VR *E. faecium* bacteremia) were then matched to controls (patients with VS *E. faecium* bacteremia) on the basis of age, date and unit of hospitalization, severity of illness, and co-morbidities, including need for dialysis, immunosuppression, history of organ transplant, HIV status, same-hospitalization surgery, and presence of a prior oncologic diagnosis. The following factors were associated with increased risk of developing VR *E. faecium* bacteremia: exposure to third-generation cephalosporins (OR 3.69, CI 1.72-7.89), vancomycin (OR 3.57, CI 1.62-7.84), anti-anaerobic agents (OR 2.36, CI 1.11-5.01), immunosuppressive agents (OR 3.57, CI 1.62-7.84), hospitalization in an ICU (OR 1.69, CI 0.82-3.51), history of organ transplant (OR 2.00, CI 0.74-5.41), and need for dialysis (OR 1.67, CI 0.70-3.97). Patients with VR *E. faecium* bacteremia were more likely to die than patients with *E. faecium* bacteremia prior to controlling for severity of illness (64.7% vs. 44.4%, OR 2.29, CI 1.10 – 4.79). After matching cases to controls on a 1:1 basis to control for severity of illness and other variables, the difference in mortality rates was no longer statistically significant (57.1% vs. 45.2%, OR 1.61, CI 0.68-3.82).

Acknowledgements:

Thank you to Louise-Marie Dembry, MD, my thesis advisor, who was always available and always happy to share her valuable time helping me along with this project. She provided just the right amount of guidance to point me in the right direction while allowing me to maintain control of the project.

Also, Ms. Sue Roberts, from Medical Records at Yale-New Haven Hospital, was very helpful in finding all relevant charts (with their many volumes) given just the date during a hospitalization. Patricia Reagan, PhD, provided important advice on how best to analyze the data. Helen Schweidler, of the Clinical Microbiology Laboratory at Yale-New Haven Hospital, was obliging in providing reports of patients with enterococcal bacteremia, and doing so very quickly.

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Introduction

The problem of antibiotic resistance is a significant one for the practice of medicine, and hospitals often devote substantial resources to limiting the proliferation and spread of antibiotic resistant organisms. Excess costs incurred by hospitals as a result of infections caused by the six most common antibiotic-resistant nosocomial infections were estimated to be *$1.3 billion / year* (in 1992 dollars) by the Office of Technology Assessment of the US Congress (1). Further, this estimate included only those expenses encountered in the hospital, and did not encompass costs such as follow-up care, missed workdays and other related expenditures (1). Organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Pseudomonas aeruginosa* are among the most concerning pathogens that require significant resources to control. In addition, enterococci have emerged as common nosocomial pathogens in recent years, becoming the second most common cause of nosocomial urinary tract infections and the third most common cause of nosocomial bacteremia (2). While possessing innate resistance to cephalosporins and many other antibiotics, the *Enterococcus* genus has only recently been elevated to the level of a more significant nosocomial pathogen when it developed resistance to vancomycin.

Vancomycin-resistance enterococci (VRE) were first reported in Europe in 1986 (3). By 1988, isolates of VRE had appeared in the United States, and began disseminating quickly. Between January of 1989 and March of 1993, the percentage of VRE isolates reported to the National Nosocomial Infections Surveillance (NNIS) system increased by a factor of twenty, growing from 0.3% to 7.9% of all enterococcal isolates (4). Even more striking, by 1998, more than 20% of all ICU enterococcal isolates reported to the NNIS were resistant to vancomycin (5). The rapid increase in prevalence of a multiply resistant organism for which there are few therapeutic options demanded attention, but it was unclear whether enterococci had actually become more virulent.

At Yale-New Haven Hospital, recognition of the first strain of vancomycin-resistant enterococci occurred in 1992. The number of vancomycin-resistant enterococcal isolates (the vast majority of which were strains of *E. faecium*) increased over the next two years. The spread of resistant organisms prompted the institution of screening (through rectal or peri-rectal cultures) and subsequent isolation of patients who were positive for VRE. Beginning in 1994, this screening was directed towards patients in the medical intensive care unit and those on patient care units with a high percentage of dialysis and HIV patients, as these units were found to have the highest prevalence of VRE based on surveillance. In 1998, the population screened for VRE was expanded to include the oncology unit, based on an increase in VRE colonization noted via periodic prevalence culture surveys. In spite of these interventions, the number of patients with VRE bacteremia continued to rise through the end of the decade.

The experience at YNHH with VRE was very similar to those at hospitals elsewhere in the country. One of the concerns over this organism's rapid spread was the loss of vancomycin as an effective pharmacologic agent for enterococcal infections. However, clinicians remained (and remain, to this day) divided on the significance of vancomycin-resistant enterococci, particularly when part of polymicrobial infections. Enterococci are not considered nearly as pathogenic as *S. aureus* or *P. aeruginosa*. However, there has been concern that VRE could pass vancomycin resistance onto an organism such as MRSA, thereby eliminating the primary therapeutic option for a formidable pathogen. While *S. aureus* with reduced susceptibility to vancomycin were documented in the U.S. in 1997, it was not through the same mechanism as employed by enterococci (6). Nonetheless, concerns over the *Enterococcus* species itself – which includes organisms that are typically found in the normal flora of our intestine – persist. But, whether we are justified in devoting significant hospital resources to combat the

spread of an organism that is prevalent, but of unknown pathogenicity, is a question that remains unanswered.

Background

As gram-positive cocci, enterococci were originally classified as Group D streptococci, and were differentiated from other *Streptococcus* species by their ability to grow on 6.5% NaCl. As a whole, enterococci are remarkably hardy, capable of growing in medium with a pH of 9.6 or containing 40% bile, as well as at temperatures as low as 10°C and as high as 45°C (7). In the 1980s, when it was determined that enterococci differed from streptococci not only in their ability to grow under harsher conditions but also genetically, they were reclassified under their own genus (2).

Clinical enterococcal isolates are most commonly determined to be either *Enterococcus faecalis*, which comprises roughly 80 – 90% of isolates, or *Enterococcus faecium*, comprising an additional 5 – 15% (2). Laboratories differentiate between the two species based on their differing abilities to ferment sugars. *E. faecium* is able to ferment arabinose, while *E. faecalis* is unable to do so. In addition to the most common species, approximately 5% of isolates belong to the other species of *Enterococcus*, which include *E. gallinarum, E. casseliflavus, E. durans, E. avium,* and *E. raffinosis* (2). These different species of enterococci demonstrate varying mechanisms of antibiotic resistance. As discussions of the relevance of enterococci are based in no small part on the organism's resistance, this subject merits further explanation.

Resistance in enterococci

Just as enterococci have been known to be a causative agent of endocarditis for decades, certain aspects of its inherent resistance to antibiotics have been known for decades as well. It was observed in the 1940s that treating endocarditis caused by

Enterococcus species with penicillin resulted in worse outcomes than streptococcal endocarditis treated with the same regimen (8). Further investigations since that time have shown enterococci to be resistant to cephalosporins, anti-staphylococcal penicillins, aminoglycosides at low-levels, and frequently to fluoroquinolones. It has been postulated that the emergence of enterococci as nosocomial pathogens is related to the increasingly frequent use of cephalosporins over the same time period and consequent selection for enterococci (2).

The mechanisms of antibiotic resistance employed by *Enterococcus* species are varied. With regard to penicillin resistance, *E. faecalis* is known to employ β-lactamases while *E. faecium* produces different penicillin-binding proteins (8). Concerning the latter mechanism, one common penicillin-binding protein (PBP) expressed by *E. faecium*, PBP 5, demonstrates much lower affinity for penicillin than do other enterococcal PBPs. Predictably, the loss of the ability to produce PBP 5 by *E. faecium* has been shown to dramatically increase penicillin susceptibility in that species (2).

Enterococci also demonstrate varying mechanisms of resistance to aminoglycosides. In fact, even resistance to gentamicin and streptomycin are mediated by different mechanisms. Resistance to one is not a certain indication that the organism will be resistant to the other (2). Resistance to gentamicin, kanamycin, tobramycin, and amikacin occur through the actions of an acetyltransferase enzyme that inactivates these aminoglycosides (2). Another enzyme, streptomycin adenyltransferase, mediates resistance to streptomycin. In addition, moderate-level resistance to all types of aminoglycosides can occur as a result of low cell-wall permeability (which can be overcome by concomitant administration of penicillins) (2).

As is the case in its resistance to other antibiotics, enterococcal resistance to vancomycin can occur in a variety of ways. The five phenotypic types of vancomycin resistance in enterococci have been designated VanA, VanB, VanC, VanD, and VanE.

The first two types, VanA and VanB, result from the presence of genes that enterococci do not possess by nature (2). The first gene, VanA, mediates inducible resistance to both vancomycin and teicoplanin, another glycopeptide antibiotic (2). Such resistance can be induced not only by exposure to glycopeptides, but also by antibiotics such as bacitracin and polymyxin B (2). This gene allows enterococci to produce cell wall precursors that terminate in D-Ala-D-Lac, instead of the D-Ala-D-Ala sequence that is bound and inhibited by vancomycin (2). Essential to the resistance mediated by VanA are two other genes that allow for key steps in mediation of resistance. VanH produces an enzyme that catalyzes production of D-Lac, which is incorporated into cell-wall precursors. In addition, VanX produces a peptidase that hydrolyzes D-Ala-D-Ala cell wall precursors, but has no activity in degrading D-Ala-D-Lac (2). Further, two other genes, designated VanR and VanS, control expression of the Van H and Van X genes. The protein-products of these genes increase expression of the VanHAX cluster in the presence of antibiotics such as vancomycin (2). Of note, this entire gene complex resides on a transposon in enterococci, thus permitting transmission of the resistance genes to other bacteria.

The VanB gene mediates resistance to vancomycin, but enterococci expressing this gene are still susceptible to teicoplanin. The protein produced by this gene also mediates the production of D-Ala-D-Lac, once again replacing the target of vancomycin by providing different cell-wall precursors (2). This mechanism of resistance also requires homologues of VanH, VanX, VanR, and VanS. This system is induced by vancomycin but not by teicoplanin (2).

Both the VanA and VanB genes can be found on mobile genetic material that can be transferred from one *Enterococcus* to another (2). It is precisely this potential for mobility that allows resistance to be transferred to other enterococci, or possibly to other similar microbes such as *S. aureus*. Such transmission has been demonstrated in the

laboratory, where *E. faecalis* passed on genes coding for vancomycin resistance to *S. aureus*. This transfer was demonstrated both in vitro, on filter paper, and also in vivo, after strains of the two bacteria were mixed and incubated on the skin of mice (9). Fortunately, the VanB gene is usually located on the main enterococcal chromosome, and in this case is not readily transferable. Most likely as a consequence of its ability to be transmitted from one bacterium to another, VanA is the mode of resistance most frequently found in the United States (2).

Other variants of vancomycin resistance are far less common. VanC typically mediates low levels of vancomycin-resistance, and is found in enterococcal species less frequently observed in the hospital setting (*E. gallinarum, E. casseliflavus, E. flavescens*) (2). VanD is a rare mode of resistance observed in *E. faecium*, and works by a mechanism similar to VanA and VanB. However, it is found on the main chromosome, and thus is not transferable (2). Finally, VanE has been documented in *E. faecalis*, and resembles resistance mediated by the VanC gene (2).

Related research

Numerous studies have investigated the epidemiology of vancomycin-resistant enterococci. A complete understanding of the patterns of resistance just described can allow one to predict some of the findings. For example, since the most common form of vancomycin resistance is mediated by VanA, the expression of which is increased in the presence of vancomycin, one can correctly surmise that administration of vancomycin selects for carriage of VRE. This expectation has been confirmed in several studies (10, 11, 12, 13, 14,15, 16). Furthermore, given that enterococci are inherently resistant to cephalosporins, administration of cephalosporins (particularly those of the third generation) is associated with emergence of VRE (10, 11, 12, 14).

Most studies concerning VRE have shown that greater length of hospitalization prior to the development of bacteremia, ICU care, and increasing severity of illness are all risk factors for infection with VRE (12, 14, 17, 18, 19, 20). One study correlated use of central venous catheters with VRE bacteremia (19). Another found a history of AIDS / HIV and liver transplant to be highly associated with VRE bacteremia as compared to VSE bacteremia (11). An association between dialysis and vancomycin-resistant *E. faecium* infection or colonization was established in one study (20), while other studies made a more specific association between hemodialysis and VRE bloodstream infections (16, 21).

While previous studies cite common factors for the development of VRE infections, they have not been conclusive in determining whether vancomycin-resistance independently affects outcome of patients with enterococcal bacteremia. One reason for this persistent uncertainty is the heterogeneity of published investigations. Studies have been difficult to compare because investigators have looked at very different patient populations; some have focused only on ICU patients, others on transplant patients, and others on the entire hospital population. A focus on different *Enterococcus* species – *E. faecalis* and *E. faecium* vs. *E. faecium* alone – also limits direct comparison, as bacteremia with *E. faecium* has been associated with worse outcome than bacteremia with *E. faecalis* (12, 22). In addition, the use of different control populations (a study by Edmond used patients without bacteremia (23), while most others used patients with VSE bacteremia), and varying attempts to correct for the effect of severity of illness on outcome inhibits direct comparison of available studies.

Among the studies using various populations, enterococcal species, and matching techniques, some failed to show higher mortality among patients with VRE as opposed to VSE bacteremia. Mainous et al examined enterococcal bacteremia (both *E. faecium* and *E. faecalis*) in patients in the surgical intensive care unit. The study found

no difference in mortality between 10 patients with VRE bacteremia and 31 patients with VSE bacteremia; also, the mortality rate for VRE bacteremia was similar to the mortality rate for bacteremia due to other organisms (41% vs. 41.7%) (14). Another study compared 93 patients with VRE bacteremia to 101 patients with VSE bacteremia, the latter selected because of positive culture dates close to the patients with VRE bacteremia. The authors found the APACHE II score to be most closely associated with death; vancomycin-resistance did not have a statistically significant effect on outcome (19). A third study compared 72 patients with VRE bacteremia to 188 patients with VSE bacteremia (neither group was limited with regard to enterococcal species). The authors concluded that vancomycin resistance was more of a marker for severe illness rather than a predictor of mortality (16).

Among the studies which did find that vancomycin-resistance contributed to mortality, one was a comparison of 53 cases with VRE bacteremia matched to 53 controls with VSE bacteremia on the basis of severity of illness, age, and hospital unit. The study included both *E. faecium* and *E. faecalis* bacteremia, and found vancomycin-resistance to be an independent predictor of mortality (17). In a study performed across twenty-two institutions, 150 patients with VRE bacteremia were compared to 150 patients with VSE bacteremia. The study examined both *E. faecium* and *E. faecalis,* and demographic data were similar in the two groups. APACHE II scores were used in multivariate analysis of the data, but comparative APACHE II scores of the two groups were not reported. Results from this comparison indicated that vancomycin-resistance was associated with higher mortality, as patients with VRE bacteremia were nearly twice as likely to die than those with VSE bacteremia (52% vs. 27%, $p \leq 0.05$) (11). Finally, another study matched 27 patients with VRE bacteremia to 27 patients without bacteremia based on age, underlying illness, and other factors. The study determined that the bacteremic patients were twice as likely to die as those without bacteremia;

however, they noted that the mortality rate for their group with VRE bacteremia was comparable to the rate of those with VSE bacteremia in other studies (23).

Among the studies that exclusively examined *E. faecium*, the primary cause of VRE infections, results are similarly mixed as to whether vancomycin-resistance is a predictor of worse outcome. Garbutt et al. performed a retrospective study of all patients with *E. faecium* bacteremia at Barnes-Jewish Hospital in St. Louis over a 28-month period. The main cohort of patients consisted of 23 patients with VSE and 46 patients with VRE who all had clinically significant bacteremia (defined as two positive blood cultures, or one positive blood culture with another site, other than stool, positive for *E. faecium*). Severity of illness was evaluated using APACHE II scores, the Organ System Failure Index, the Chow index (referred to as the Korvick Index in this study – a severity of illness score established and validated by previous studies of bacteremic patients), and the Systemic Inflammatory Response Syndrome classification scheme. The authors concluded that while each of their severity of illness scores was positively associated with risk of death, vancomycin-resistance was not independently associated with mortality. However, the authors postulated that a larger sample size might lead to a different conclusion (24).

Stosor et al. completed a retrospective investigation of patients with *E. faecium* bacteremia at Northwestern Memorial Hospital between 1992 and 1995. Medical records of fifty-three patients were reviewed, of which 32 patients had VSE bacteremia, and 21 had VRE bacteremia. The authors used the fore-mentioned Chow Index to control for severity of illness. On statistical analysis, the two groups had similar severity of illness scores, but the mortality rate differed significantly (76% mortality for patients with VRE bacteremia, vs. 41% for patients with VSE bacteremia, p = 0.009) (15). This difference in mortality was attributed to vancomycin-resistance given the otherwise similar characteristics of the two groups. One shortcoming of this study was the

inclusion of nine patients with bacteremia that did not meet the author's own definition of clinical significance (requiring a minimum of two positive cultures, or one positive culture with a documented source of infection). As no deaths were attributed to VRE bacteremia if the bacteremia did not meet the definition of clinical significance (15), this may have biased the outcome of the study. Moreover, as six of these patients were in the VSE group and the numbers in each group were very small to start with, the significance of the outcome of this study is questionable.

A third study examined consecutive patients on a liver transplant service who developed *E. faecium* bacteremia. The authors compared 54 patients with VRE bacteremia to 48 patients with VSE bacteremia, and concluded that vancomycin-resistance was an independent predictor of mortality (18). However, the study did not attempt to control for severity of illness. While all patients were on a liver-transplant service, examination of the patients' co-morbidities showed differences between the numbers of transplant patients in each group (and thus patients who were actively immunosuppressed), the number of patients who developed bacteremia in the ICU (patients who were probably more severely ill), and the number of patients who were on hemodialysis or mechanical ventilation. When these differences are considered, the group with VRE bacteremia appears to have been more seriously ill than those with VSE bacteremia.

Current Study

Purpose

As mentioned above, while many studies have examined the effect of vancomycin-resistance on outcome of patients with bacteremia, there is no general consensus on whether vancomycin-resistance is an independent predictor of mortality for these patients. This question demands a carefully structured investigation capable of

providing a credible answer. Since the majority of vancomycin-resistant enterococci are *E. faecium*, we chose to investigate the outcome of bacteremia specifically caused by this species. Furthermore, as severity of illness undoubtedly has an effect on mortality, we paid particular attention to this variable. We also tried to avoid a shortcoming of previous smaller investigations by including a larger number of VRE cases and controls, electing to investigate a ten-year period at Yale-New Haven Hospital. Determining whether vancomycin-resistance plays a significant role in the outcome of enterococcal bacteremia may permit the hospital to appropriately allocate limited resources in controlling the spread of antibiotic-resistant pathogens.

The hypothesis to be tested is as follows: Vancomycin-resistance does not have an independent effect on mortality of patients with *E. faecium* bacteremia after controlling for severity of illness.

Patients and Methods

The study is a retrospective, case-control analysis of patients with *Enterococcus faecium* bacteremia at Yale-New Haven Hospital, a 944-bed university-affiliated, tertiary care, teaching hospital. Dr. Dembry obtained a list of all patients with enterococcal bacteremia between July 1992 and October 2002 from the Clinical Microbiology Laboratory. All patients greater than 2 years of age who had clinically significant bacteremia were included. Clinically significant bacteremia was defined as positive blood cultures from two different sites, or one set of positive cultures with another site of enterococcal infection (urinary tract infection, wound infection, etc.). Rectal swabs and sputum samples positive for VRE were not considered as sites of infection, and were not included based on our definition. Bacteremia was classified as either vancomycin-sensitive enterococci (VSE) or vancomycin-resistant enterococci (VRE), with the threshold for defining an organism as resistant to vancomycin set at an MIC ≥ 32 μg/mL.

In addition, for patients with multiple episodes of bacteremia, only the first instance was considered in our study.

After examining medical records to determine whether patients met inclusion criteria (patient age and clinically significant bacteremia), I reviewed the records for the following: gender and ethnicity; antibiotics received and the duration of treatment, both before and after the documented bacteremia; co-morbidities, including history of cancer, HIV, transplant, renal failure requiring dialysis, and same-admission surgery; exposure to immunosuppressive agents prior to the development of bacteremia; severity of illness using the Korvick Scale, based on the patient's condition on the day blood cultures were drawn; and survival outcome (discharge or death) for that hospitalization.

The Korvick Scale has been validated in prior studies as an effective measure of severity of illness in patients with bacteremia (25, 26). Point assignments for the criteria considered by the Korvick Scale are listed in Table 1. Points assigned in each category are totaled to create the overall severity of illness score, with the possible total score ranging from 0 to 14 points.

Table 1. Korvick Scale

Mental Status	
Normal	0
Disoriented	1
Stupor	2
Coma	4
Patient Temperature	
< 99.8°F	0
≥ 99.8°F and < 104°F	1
≥ 104°F	2
Blood Pressure	
Unchanged from baseline	0
Systolic drop > 20mm Hg, diastolic drop > 10mm Hg, or requiring pressors	2
Mechanical Ventilation	
N/A	0
Required	2
Cardiac Arrest	
N/A	0
Present	4

While the Korvick Scale measures the patient's severity of illness on the day of diagnosis of bacteremia, it does not reflect each patient's co-morbid illnesses. However, since this was a retrospective study and many subjects were hospitalized outside of the ICU, the thorough documentation that would have made it possible to use other severity of illness scores (such as the APACHE II scale) was not available.

Culture isolates were determined to belong to *Enterococcus* species by the Clinical Microbiology Laboratory based on their ability to grow on 6.5% NaCl and on bile-esculin agar. Isolates were further characterized as *E. faecium* based on their ability to ferment arabinose. In addition, antimicrobial sensitivity was assessed by disk diffusion method according to NCCLS guidelines.

Patients were considered to be immunosuppressed if they were actively receiving chemotherapeutic agents, were on a post-transplant immunosuppressive regimen, or received an equivalent of at least 20mg per day of prednisone for a minimum duration of five days, prior to the episode of bacteremia. Also, mortality was evaluated only in the context of the same hospitalization as the bacteremia. Patients who were discharged to inpatient hospice (including 4 patients who had VRE bacteremia, and 2 patients who had VSE bacteremia) were considered to have died during their hospitalization.

I performed the statistical analysis using EpiCalc 2000, version 1.02. Statistical significance was assessed using the chi-squared test or Fisher's exact test for dichotomous variables and with the Student's t test for ordinal variables. P values ≤ 0.05 were considered to be statistically significant.

In total, 85 patients were found to have clinically significant vancomycin-resistant (VR) *E. faecium* bacteremia between August 1992 and December 2000, and 45 patients were found to have clinically significant vancomycin-sensitive (VS) *E. faecium* bacteremia between August 1992 and October 2002. The time period for identification

of VS *E. faecium* bacteremia cases was extended in order to find an adequate number of controls. (Of note, the treatment of bacteremia with VS *E. faecium* was unchanged, so this measure would be unlikely to bias results in any way). Cases were defined as patients who had clinically significant VR *E. faecium* bacteremia, and controls were patients with clinically significant VS *E. faecium* bacteremia during the aforementioned periods.

Cases were matched with controls on the basis of severity of illness, age, nursing unit, date of positive culture, and co-morbidities including need for dialysis, immuno-suppression, history of organ transplant, HIV status, same-hospitalization surgery, and prior oncologic diagnoses. The matching system was similar to techniques used in prior studies of bacteremia and fungemia (17, 27, 28), and was weighted with the most important match characteristics being similar severity of illness (SOI) scores, dates of hospitalization (to ensure comparable treatment), and patient ages. Other characteristics on which the matching was based included nursing unit and co-morbid illnesses. Points were allocated to each potential case-control combination in the following manner: 2 points for an SOI score within 1, and 2 more points for the same SOI; 2 points for the same gender; 2 for an age within 10 years, with an additional 2 points if the age was within 5 years; 2 points if the patients were hospitalized on the same nursing unit; 2 points if the date of positive culture was within 2 years, with an additional point if it was within 12 months; and 2 points for each of the following co-morbidities in common: end stage renal disease, HIV status, history of transplant, surgery during the index hospitalization, a prior oncologic diagnosis, or immuno-suppression. Matching of cases to controls (on a 1:1 ratio) was based on the highest score of at least 14 points. Cases that did not have a score of at least 14 for any case-control combination were excluded from the study group, as they were not similar enough to any controls.

Of note, while the goal at the outset of the study was to focus on *E. faecium* bacteremia to avoid confounding by inter-species differences, we were unsure if there would be enough controls with VS *E. faecium* bacteremia. Consequently, we identified several patients with VS *E. faecalis* bacteremia that were hospitalized at similar times on similar nursing units to patients with VR *E. faecium* bacteremia to serve as potential controls. Collection of data on these forty patients gave us valuable, firsthand insight into the differences between enterococcal species, as discussed in the results.

Results

160 patients with VR *E. faecium* bacteremia were identified in the report from the Clinical Microbiology Laboratory. Of these patients, 2 were autopsy specimens, 4 charts were unavailable, 1 patient was a newborn, and 68 did not have clinically significant bacteremia, leaving 85 patients for analysis. Of 111 patients with VS *E. faecium* bacteremia, 3 were autopsy specimens, 3 charts were unavailable, 4 later had VRE bacteremia during the same hospitalization (and were thus considered to be cases), 10 were newborns, and 46 did not have clinically significant bacteremia, leaving 45 patients for analysis.

The 85 patients with VR *E. faecium* bacteremia and 45 patients with VS *E. faecium* bacteremia were analyzed in two separate groups. The main group included all cases and controls, who by definition had *E. faecium* bacteremia. The study group included 42 cases matched on a 1:1 basis with 42 controls. 43 case patients were unable to be matched with controls due to the relative paucity of patients with vancomycin-sensitive *E. faecium* bacteremia, and 3 controls with VSE bacteremia were not similar enough to any case patients to be matched. In addition, charts for 40 patients with VSE bacteremia secondary to *E. faecalis* were reviewed (for reasons described earlier). Statistics from this population are only included in a direct comparison with the 45 patients with VS *E. faecium* bacteremia.

***VSE Bacteremia – both* E. faecium *and* E. faecalis**

Since there were not a large number of patients with VS *E. faecium* bacteremia, we considered combining patients with either VS *E. faecium* or *E. faecalis* bacteremia as controls. However, evaluation of the data from 45 patients with VS *E. faecium* bacteremia compared to 40 patients with VS *E. faecalis* bacteremia demonstrated that this would not be an appropriate comparison. Patients with vancomycin-sensitive

bacteremia fared far differently based on whether they were infected with *E. faecium* or *E. faecalis*. Although the patients with VSE bacteremia, regardless of species, were similar in demographic and other characteristics (Table 2), VS *E. faecium* bacteremia resulted in higher mortality than VS *E. faecalis* (OR 3.77, 95% CI 1.38 – 10.31).

Table 2. Demographics Among Patients with VSE

	E. faecium Bacteremia	*E. faecalis* Bacteremia	P value	OR (95% CI)
Number of patients	45	40		
Mean age (range)	51.4 (4 – 89)	60.7 (4 – 90)	0.038	
Median age	52	68		
Gender (% male)	62.5%	47.5%		
Race				
White	66.7%	72.5%		
African-American	24.4%	20.0%		
Hispanic	6.67%	7.5%		
Other	2.2%	0%		
Unknown	0 %	0%		
Severity of Illness Score (Korvick)	2.11	1.98	0.707	
Crude Mortality Rate	44.4%	17.5%		3.77 (1.38 – 10.31)

Of note, while the Korvick Scores for severity of illness were similar, the average ages of the two populations (those with different species of VSE) were statistically different. Each group included two patients under the age of 20, but both the mean and median age was higher for patients with VS *E. faecalis*. Also, the mean hospitalization time prior to bacteremia for the two groups was similar (15.9 ± 19.8 days for *E. faecium* vs. 13.5 ± 17.9 days for *E. faecalis*), suggesting that the number of nosocomial infections was comparable between the two groups.

***Main Group – All VR and VS* E. faecium**

VR *E. faecium* bacteremia first appeared at Yale-New Haven Hospital in 1992, and the percentage of *E. faecium* isolates resistant to vancomycin increased over the next several years (Fig 1). During the study period, the number of patients with clinically significant VRE bacteremia, as defined in this study, peaked in 1997.

Fig. 1. *E. faecium* bacteremia

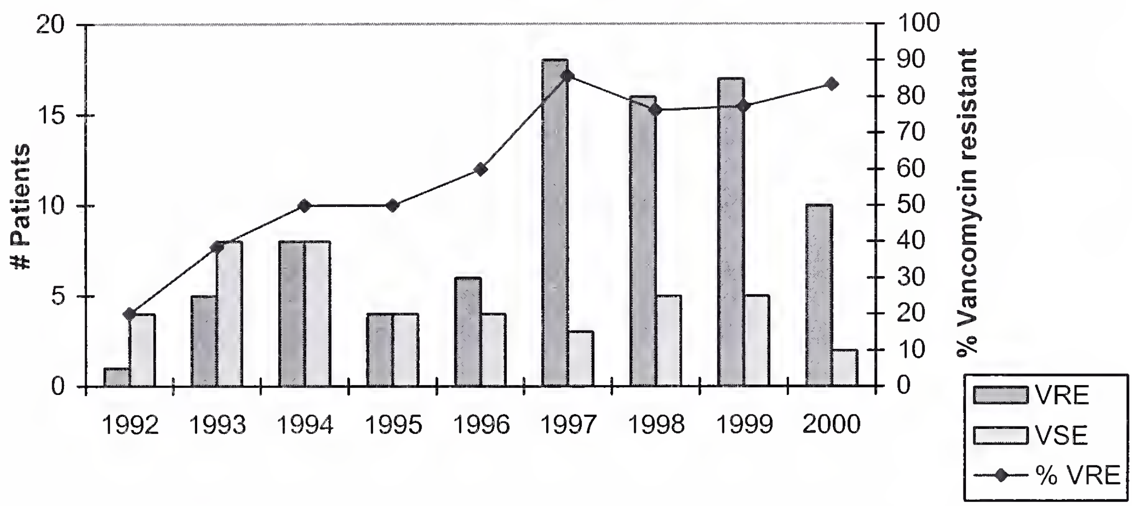

Figure 1. The bars depict the number of patients with clinically significant *E. faecium* bacteremia (blood cultures drawn from at least two sites, or one positive blood culture with another site of infection) that occurred each year at Yale-New Haven Hospital, 1992 – 2000. Patients are divided into those with VRE bacteremia and those with VSE bacteremia. The line reflects the percentage of all *E. faecium* bacteremias that were vancomycin-resistant during each year.

Mortality rates for both VRE and VSE bacteremia did not follow any clear trend over the study period (Figure 2). This is not surprising for the VSE population, as vancomycin was available for treatment of such infections during the entire study period. Pharmacotherapeutic options for VRE bacteremia changed towards the end of the study period, with the introduction of quinupristin-dalfopristin in September of 1999, and the approval of linezolid in April of 2000. However, these options were used for only four study patients, with mixed results. One patient treated with quinupristin-dalfopristin

survived, while another died of underlying disease after recovering from bacteremia. One of two patients treated with linezolid survived. Of note, the patient who died after treatment with linezolid had a severity of illness (Korvick) score of 4, while the other patient had a score of 2.

Chloramphenicol was the most frequent antibiotic prescribed for patients with VRE bacteremia over the course of the study, but outcome for these patients was not significantly different than those treated with other medications (including gentamicin and doxycyline, as well as linezolid and quinupristin-dalfopristin). The mortality rate for patients treated with chloramphenicol was 63.9%, while patients treated with other regimens had a mortality rate of 54.5% (p = 0.304).

Fig 2. Mortality Rate

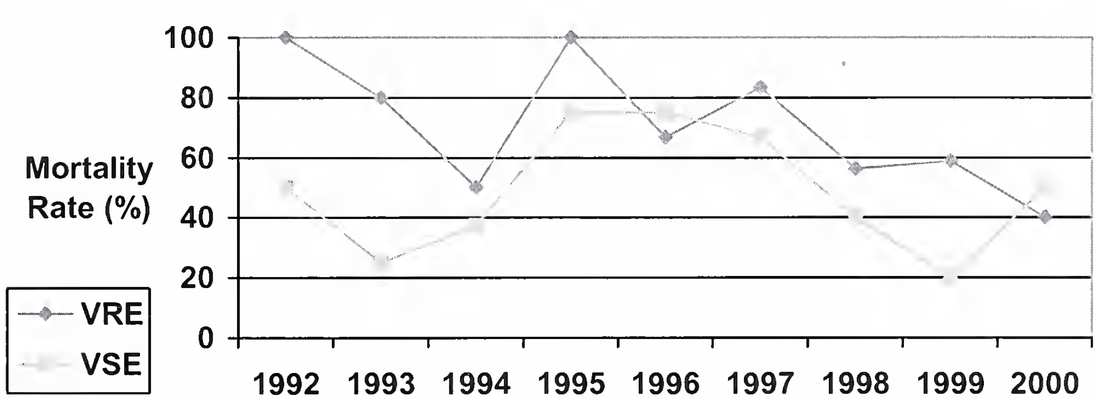

Figure 2 shows the mortality rate over time for both VRE and VSE bacteremia at Yale-New Haven Hospital. Statistics were calculated based on all clinically significant cases of bacteremia.

More than three quarters of the study population had a Korvick Score less than or equal to 3 (Figure 3). There were relatively few patients at the higher end of the Korvick scale, particularly in the group with VSE bacteremia. The highest Korvick Score in each group was 7 points (out of a possible 14 points).

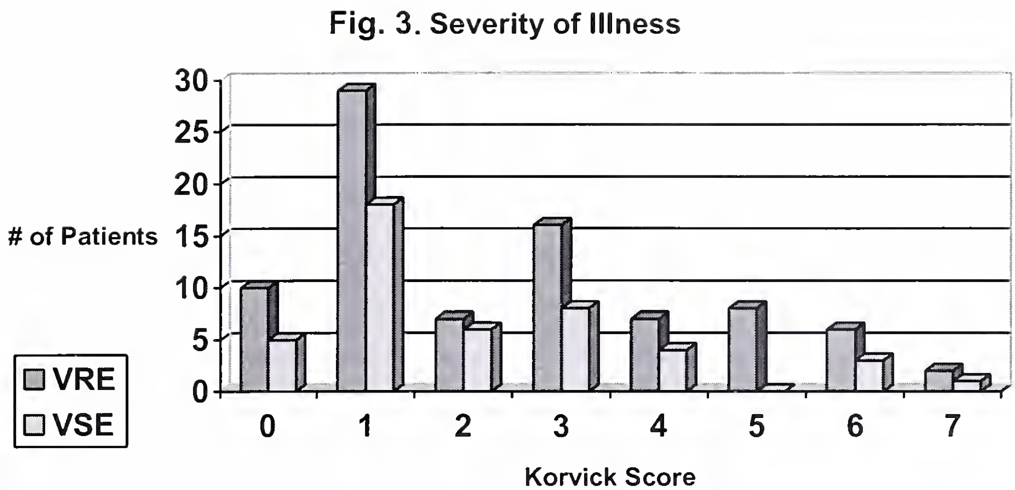

Figure 3 shows the number of patients observed for each Korvick Score, with separate totals for those with VRE vs. VSE bacteremia.

The Korvick Scale proved to be an accurate predictor of outcome in this study of patients with *E. faecium* bacteremia, particularly those with VRE. The group of patients with VR *E. faecium* bacteremia shows a relatively clear trend towards higher mortality given a higher Korvick Score (Fig. 4). The apparent dip in mortality at a score of 2 may be a consequence of the fact that only seven patients had a score of 2, providing less than half the number of observations for a score of 1 (29 patients) or 3 (16 patients). Also, more than 80% of patients with VR *E. faecium* bacteremia and a Korvick Score of at least 5 died; one patient with a score of 6 was the only survivor at this end of the Korvick Scale.

The trend towards higher mortality with increasing Korvick Score is less definite in patients with VSE bacteremia. The relatively few patients with both VSE bacteremia and high Korvick Scores may have precluded us from noting a clearer trend towards higher mortality in this population.

Fig 4. Mortality by Severity of Illness

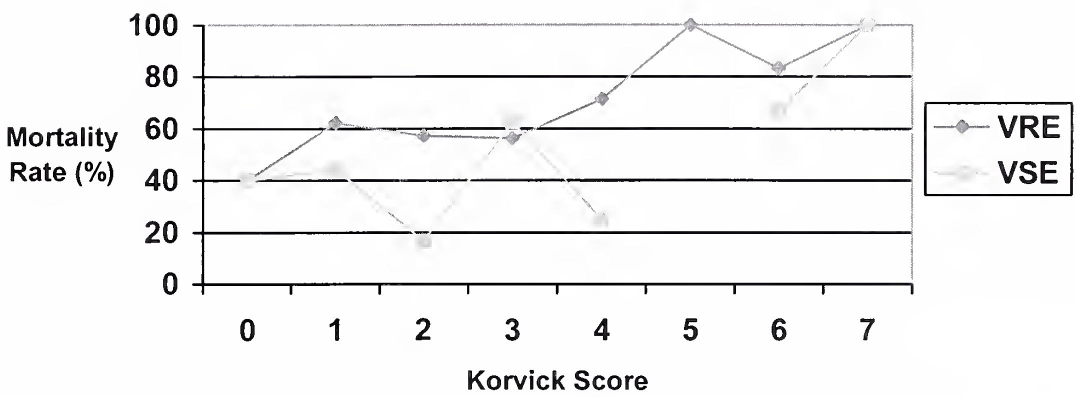

Figure 4 shows the mortality rate at each severity of illness score. Mortality rates were calculated for patients with clinically significant VRE or VSE bacteremia.

Among the main group, the demographic data was similar between patients with VRE bacteremia and those with VSE bacteremia (Table 3). The VRE group was slightly older than the VSE group, but this difference was not statistically significant. In addition, the VSE group had a slightly higher percentage of males (64.4% as opposed to 56.5%), but again, this difference was not statistically significant. Prior to controlling for severity of illness, patients with VRE bacteremia had a higher crude mortality rate, 64.7% vs. 44.4%. In this analysis, VRE bacteremia was associated with mortality by an odds ratio of 2.29 (95% CI 1.10 – 4.79). These two groups also had differing severity of illness scores, with the average Korvick score being 2.46 in the VRE group, and 2.11 in the VSE group; however, this difference was not statistically significant ($p = 0.314$).

The two groups had similar proportions of patients with polymicrobial bacteremia, 32.9% in the VRE group compared to 40.0% in the VSE group. On the other hand, the percentage of patients in each group with persistent bacteremia (at least one blood culture drawn on a different day was positive) was much higher in the VRE group – 60.0% of patients had persistent VRE bacteremia, while 35.6% of patients had persistent

VSE bacteremia. The association between VRE and persistent bacteremia was statistically significant (OR 2.72, 95% CI 1.29 – 5.75).

Table 3. Characteristics of Patients with *E. faecium* Bacteremia

	VRE Bacteremia	VSE Bacteremia	P value	OR (95% CI)
Number of patients	85	45		
Age	55.1	51.4	0.250	
Gender (% male)	56.5%	64.4%		0.72 (0.34 – 1.51)
Race				
White	70.6%	66.7%		
African-American	21.2%	24.4%		
Hispanic	5.88%	6.67%		
Other	1.18%	2.2%		
Unknown	1.18%	0 %		
Severity of Illness Score (Korvick)	2.46	2.11	0.314	
Polymicrobial Bacteremia	32.9%	40.0%		0.76 (0.36 – 1.62)
Persistent Bacteremia	60.0%	35.6%		2.72 (1.29 – 5.75)
Crude Mortality Rate	64.7%	44.4%		2.29 (1.10 – 4.79)

As previously mentioned, all patients were considered to have clinically significant bacteremia in the presence of two positive blood cultures from different sites, or one positive blood culture with another documented site of infection. Of the patients with only a single positive blood culture, the additional site of infection varied (Table 4). Additionally, 28 patients with one positive blood culture for VRE were not considered to have clinically significant bacteremia although they were documented as being colonized with VRE by positive rectal swabs. The total number of clinically significant cases of bacteremia was significantly higher in the VRE group than the VSE group (53.1% vs. 40.5%, p = 0.0414).

Table 4. Concurrent Sites with VRE

	VRE	VSE	P value
Patients with ≥ 1 positive blood culture during study period	160	111	
Patients with 2 or more positive blood cultures	65	28	0.0086
Patients with only 1 positive blood culture, and a positive culture from:			
Urine	8	8	
Wound	3	2	
Ascites	3	0	
Pleural fluid	2	0	
CSF	0	1	
Bile	3	0	
Catheter tip	1	5	
Autopsy	0	1	
Sputum	3	0	
Stool / Rectal swab *	28	0**	
Total considered significant	85 (53%)	45 (41%)	0.0414

*These sites were not considered significant sites for purposes of the case definition in patients with only one blood culture positive for VRE or VSE.
**Rectal swab cultures were reported as either positive or negative for VRE; the Clinical Microbiology Laboratory did not report the presence of VSE in stool, rectal, or peri-rectal cultures.

Next, several risk factors analyzed in previous studies of enterococcal bacteremia were reviewed in our patient population. Many of the same trends were found in our patient population (Table 5). Patients with VRE were more likely to have been exposed to a minimum of three days of either 3rd generation cephalosporins (OR 4.13, 95% CI 1.91 – 8.93) or vancomycin (OR 3.57, 95% CI 1.62 – 7.84) prior to developing bacteremia. The association between vancomycin and VRE is even more pronounced when considering all patients who had one or more doses of vancomycin (OR 5.91, 95% CI 2.67 – 13.09). Also, patients exposed to anti-anaerobic agents (a minimum of three days of clindamycin or metronidazole) were at a higher risk of becoming infected with VRE (OR 2.36, 95% CI 1.11 – 5.01). Finally, exposure to

fluoroquinolones was associated with a significantly increased risk of infection with VRE; 48.2% of the VRE group received fluoroquinolones prior to developing bacteremia vs. 20.0% of the VSE group (OR 3.73, 95% CI 1.60 – 8.68).

Table 5. Antibiotic Exposures

	VRE Bacteremia	VSE Bacteremia	OR (95% CI)
Number of patients	85	45	
% of patients receiving:			
Cephalosporins (CP)	72.9%	42.2%	3.69 (1.72 – 7.89)
3rd Gen. CP	67.1%	35.6%	4.13 (1.91 – 8.93)
Vancomycin	56.5%	26.7%	3.57 (1.62 – 7.84)
Clindamycin or metronidazole	54.1%	33.3%	2.36 (1.11 – 5.01)
Penicillin class	36.5 %	35.6%	1.04 (0.49 – 2.21)
Aminoglycosides	30.6%	17.8%	2.04 (0.83 – 4.98)
Fluoroquinolones	48.2%	20.0%	3.73 (1.60 – 8.68)
Other antibiotics	49.4%	31.1%	2.16 (1.01 – 4.63)

Further analysis of the data yielded additional risk factors for VRE bacteremia (Table 6). Patients with VRE bacteremia were significantly more likely to have been in the hospital longer prior to the onset of bacteremia (27.2 days vs. 15.9 days, p = 0.0193). With regard to the total number of days of hospitalization, there was a slight difference between the two groups, 52.6 days in the VRE group vs. 41.4 days in the VSE group, however this difference was not statistically significant (p = 0.121). The number of hospital days after the first positive blood culture for enterococci was identical for the two groups, 24.6 days for patients with either VSE or VRE bacteremia. However, the apparent equality in the length of stay after diagnosis of bacteremia is a result of the number of patients with VRE bacteremia who died shortly after documentation of positive blood cultures; for survivors, the average number of days in the hospital post

bacteremia was 31.1 days for the VRE group and 23.0 days for the VSE group. This difference was not statistically significant ($p = 0.245$).

In addition, patients on immunosuppressive therapy were found to be at a statistically significant increased risk for VRE bacteremia (OR 3.57, 95% CI 1.62 – 7.84). Hospitalization in one of the intensive care units was associated with VRE bacteremia, but was not statistically significant (OR 1.69, 95% CI 0.82 – 3.51). Also, dialysis was associated with an increased risk of VRE bacteremia, with 29.4% of patients requiring dialysis prior to the onset of VRE bacteremia vs. 20.0% prior to VSE bacteremia. This increased risk associated with dialysis was also not statistically significant. Additionally, there was a non-significant trend for patients with a history of organ transplant to develop VRE bacteremia (OR 2.00, 95% CI 0.74 – 5.41). In this study, other co-morbidities including HIV status, surgery during the same hospitalization, and an oncologic diagnosis were not found to be associated with risk for VRE bacteremia.

Table 6. Risk Factors for VRE Bacteremia

	VRE Bacteremia	VSE Bacteremia	P value / OR (95% CI)
Number of patients	85	45	
Avg days of hospitalization before bacteremia	27.2	15.9	0.0193
Total hospital days	52.6	41.4	0.121
ICU stays	55.3%	42.2%	1.69 (0.82 – 3.51)
Dialysis	29.4%	20.0%	1.67 (0.70 – 3.97)
HIV positive	12.9%	13.3%	0.97 (0.33 – 2.81)
Transplant	23.5%	13.3%	2.00 (0.74 – 5.41)
Surgery (this hospitalization)	38.8%	42.2%	0.87 (0.42 – 1.81)
Oncologic Diagnosis	35.3%	31.1%	1.21 (0.56 – 2.61)
Immunosuppression	56.5%	26.7%	3.57 (1.62 – 7.84)

Study Group – Matched Cases and Controls

For the patients with VRE bacteremia who were successfully matched to controls with VSE bacteremia, the two populations had similar characteristics (Table 7). Furthermore, after matching on the basis of severity of illness, patient age, date of hospitalization, and co-morbidities, the mortality rates in the two groups differed somewhat, but not significantly (57.1% vs. 45.2%, OR 1.61, 95% CI 0.68 – 3.82). The average severity of illness score differed slightly between the two groups, but not significantly. With regard to other factors examined (including ICU status and co-morbidities), the two groups did not differ significantly in any category.

Table 7. Demographics, matched group

	VRE Bacteremia	VSE Bacteremia	P value	OR (95% CI)
Number of patients	42	42		
Age	50.3	53.7	0.376	
Gender (% male)	28 (66.7%)	27 (64.3%)		
Race				
White	29 (69.1%)	28 (66.7%)		
African-American	11 (26.2%)	10 (23.8%)		
Hispanic	1 (2.38%)	3 (7.14%)		
Other	1 (2.38%)	1 (2.38%)		
ICU stay	20 (47.6%)	16 (38.1%)		1.48 (0.62 – 3.52)
Dialysis	9 (21.4%)	9 (21.4%)		1.00 (0.35 – 2.84)
HIV positive	8 (19.1%)	6 (14.3%)		1.41 (0.44 – 4.49)
Transplant	11 (26.2%)	5 (11.9%)		2.63 (0.82 – 8.38)
Surgery (this hospitalization)	19 (45.2%)	17 (40.5%)		1.21 (0.51 – 2.89)
Oncologic Diagnosis	14 (33.3%)	13 (31.0%)		1.12 (0.45 – 2.79)
Severity of Illness Score (Korvick)	1.81	2.00	0.595	
Crude Mortality Rate	24 (57.1%)	19 (45.2%)		1.61 (0.68 – 3.82)

Discussion

This study supports the results of Garbutt et al. who observed that vancomycin-resistance does not have a significant effect on outcome of enterococcal bacteremia after controlling for severity of illness. A long-standing question has been whether infection with vancomycin-resistant enterococci is merely a marker of severe illness or actually portends a worse prognosis. Our study suggests the former. The relatively low pathogenicity of enterococci has never been debated. In fact, prior to the development of vancomycin resistance, it was debated as to whether enterococcal bacteremia even merited treatment (29, 30). While it was ultimately determined that patients receiving appropriate treatment had slightly improved outcomes, this debate underscores the lack of virulence of enterococci.

The development of vancomycin-resistance renewed the debate over enterococci again; does VRE lead to higher mortality than VSE? There is currently no evidence that VRE possesses virulence factors lacking in VSE to suggest differences in pathogenicity. Furthermore, several studies are concordant in demonstrating that patients who are more severely ill are more likely to develop infection with VRE (13, 16, 20, 21, 23). Likewise, the present study suggests that patients who develop VRE bacteremia are more likely to die than those developing VSE bacteremia, but only before controlling for severity of illness.

It might be postulated that mortality is increased among those with VRE bacteremia because of a lack of treatment options. This did not appear to be the case in our study. There were no pan-resistant VRE isolates encountered during review of susceptibilities, and most of the isolates were sensitive to chloramphenicol. While patients treated with chloramphenicol did not have better outcomes than patients treated with other regimens, the higher mortality rate for those patients may represent treatment bias, as the risks of chloramphenicol may have limited its use to the most severely ill

patients. Future studies are not likely to document lack of treatment options as a source of poorer outcome either, as linezolid and quinupristin-dalfopristin now provide additional options in the treatment of VRE.

Another interesting finding of our study was that *E. faecium* was associated with significantly higher mortality rates than *E. faecalis* among those patients with vancomycin-sensitive enterococcal bacteremia. This was in spite of the fact that the average age of the patients with VS *E. faecalis* was significantly higher than the patients with VS *E. faecium* bacteremia. A difference in virulence between the two species had been suggested previously. In one study, patients with VS *E. faecalis* bacteremia were compared to patients with *E. faecium* bacteremia, with the latter group containing some patients infected with VRE (12). The current study population highlights this inequality between different *Enterococcus* species, and suggests caution in interpreting results of earlier studies. Many of these studies grouped both species in the VSE category, while the VRE group was primarily composed of *E. faecium*. At Yale-New Haven Hospital, 94.1% of the patients with a blood culture positive for VRE during the study period were infected with *E. faecium*, while only 13.1% of the patients with VSE over the same time period were infected with this enterococcal species. Thus, VRE and VSE categories largely encompass different species of enterococci. Interpretation of virulence based on resistance to vancomycin requires cognizance of this fact.

A mechanism accounting for the observed difference in virulence between *E. faecium* and *E. faecalis* has not been described. Both *E. faecium* and *E. faecalis* possess cytolysins (which also act as hemolysins) and aggregation substances (which facilitate transmission of DNA, including transposons that carry genes mediating resistance). *E. faecalis* alone produces virulence factors such as proteases and hyaluronidase (31). Conversely, there is documentation of an uncharacterized substance produced only by *E. faecium* that inhibits phagocytosis in vitro (32). While

this suggests a possible mechanism for the observed difference in virulence between the two species, it is clear that the pathophysiology of enterococcal infections is not completely understood. In any event, such inter-species differences would also suggest that *E. faecium* and *E. faecalis* must be considered separate entities in investigations of enterococci, as previously suggested by Mackowiak (7).

If the premise is correct that vancomycin-resistance does not affect mortality or other clinical outcomes, the possibility that VRE is associated with increased cost of care could be reason alone to pursue aggressive (but cost-efficient) methods of containment. We did not determine the hospital costs for each patient, but the number of days spent in the hospital (a major determinant of total costs for hospitalization) was not significantly different between the VRE and VSE groups. More importantly, while the number of hospital days prior to bacteremia was significantly longer for the VRE group, the number of hospital days after documentation of bacteremia was very similar. Studies that document excess costs for VRE patients (as opposed to their counterparts with VSE) may be inappropriately attributing increased costs to VRE when it is more likely that the costs are related to patient's severity of illness. Stosor et al., for example, reported that patients with VRE bacteremia were hospitalized significantly longer before developing bacteremia as compared to those with VSE bacteremia (34.8 days vs. 16.7 days) (15). However, they attributed all differences in the cost of the hospitalizations to VRE, when in fact a significant amount of these excess costs were most likely incurred before the bacteremia.

Of course, factors other than the number of days of hospitalization can affect costs of treatment. Newer antibiotics used to combat VRE infections will certainly affect the balance of costs; both linezolid and quinupristin-dalfopristin are far more expensive than either vancomycin or chloramphenicol. Furthermore, these drugs may be necessary to treat many infections with VRE that do not involve bacteremia. At Yale-

New Haven Hospital, bacteremia alone accounts for only 25 – 30% of all VRE infections, so the number of other infections that may require these expensive drugs is significant. Clearly, the issue of whether vancomycin-resistance leads to excess costs when treating enterococcal infections requires further study.

Aside from the possibility that vancomycin-resistance may increase the cost of treating enterococcal infections, another important reason to continue efforts in preventing the spread of VRE would be to prevent the spread of resistance to other organisms. While the transmission of resistance from *Enterococcus* species to organisms such as *S. aureus* had been previously documented both in vitro and in vivo, it had never been reported to occur outside of a laboratory until very recently. In July, and again in October of 2002, the CDC reported isolates of *S. aureus* that were resistant to vancomycin. The first case involved a dialysis patient, who was documented to have both VRE and MRSA infections prior to development of vancomycin-resistant *Staphylococcus aureus* (VRSA) (33). This patient had also received several courses of vancomycin therapy. Fewer details were revealed concerning the second patient, but it is assumed that VRSA developed after conjugation between MRSA and VRE, as this isolate (like the previous specimen) was documented to possess the VanA phenotype (34).

If the transmission of vancomycin resistance from *Enterococcus* species to other organisms were common, development of VRSA would likely have occurred much sooner after the appearance of VRE. Furthermore, one would expect that *E. faecalis* would quickly become the predominant species of VRE (as it is in VSE) if transmission of resistance through conjugation happened frequently. The fact that *E. faecium* comprises a high percentage of VRE isolates, but only a fraction of VSE isolates suggests that such transfer probably remains fairly rare. However, even if conjugation leading to the spread of resistance is rare, the transmission of vancomycin resistance is

no longer just a laboratory phenomenon, and must be considered in any recommendations concerning control of VRE. The development of vancomycin-resistance in a pathogenic organism such as *S. aureus*, which can be deadly even in immunocompetent hosts, eliminates the main therapeutic option for MRSA infections. Leaving only second-line therapy for such infections would surely lead to increased mortality due to such infections. For this reason alone, we should continue efforts to contain VRE, in spite of its low level of virulence. Such efforts can be focused on limiting risk factors for transmission of VRE that have been established in other studies and confirmed in this population, and also on general measures that serve to prevent transmission of all resistant microbes.

Antibiotics

This study confirmed that exposure to certain antibiotics is a risk factor for infection with VRE. In particular, administration of cephalosporins, especially those of the third generation, clearly leads to greater risk of VRE infection. It has long been known that enterococci are intrinsically resistant to cephalosporins, and it is believed that eliminating the microbial competition by other organisms allows for the proliferation of enterococci. Furthermore, this study confirmed parenteral vancomycin as a risk factor for the development of VRE. As mentioned previously, vancomycin and other glycopeptides are key to the induction of VanA resistance genes, thus selecting for the growth of enterococci possessing the genes for resistance.

We also found that exposure to antibiotics with activity against anaerobes (metronidazole and clindamycin) was associated more strongly with VRE than VSE infection. This association received comment in previous studies (19, 24). The relationship we observed between exposure to fluoroquinolones and development of VRE was also noted in a previous study (13). Enterococci are known not only to

develop, but also to possess innate resistance to fluoroquinolones, so this association is not surprising. Furthermore, *E. faecium* has higher minimum inhibitory concentrations for fluoroquinolones than *E. faecalis* (MIC_{90} > 100 μg/mL for the former and MIC_{90} = 3.12 μg/mL for the latter in one study) (35).

These associations notwithstanding, a direct link between antibiotic exposure and development of VRE infections has not been proven. Administration of antibiotics to which enterococci are resistant could plausibly lead to overgrowth of enterococci on the skin or in bowel. Examination of skin and intestinal carriage of enterococci in patients with VRE bacteremia showed that 86% of these patients had VRE on their skin, and 100% had positive rectal cultures indicating intestinal carriage (36). VRE carriage on the skin likely increases the likelihood that an IV catheter would provide a portal of entry into the bloodstream for VRE. We did not collect data on IV catheter use in our cohort of patients, but most of them (a significant proportion of whom were in the ICU) likely had some type of IV catheter. Previous studies have documented an association between central venous catheters and VRE bacteremia (19, 24).

Another possible mechanism of progression from bacterial overgrowth to bacteremia could be through passage across the intestinal epithelium. Mice fed an oral inoculum of *E. faecalis* and exposed to antibiotics without activity against enterococci were shown to develop disseminated infection with *E. faecalis*, including infection of the mesenteric lymph nodes, liver, and spleen (37). If the findings in mice can be extrapolated to humans, enterococci may be capable of translocating across an intact intestinal mucosa, using the portal venous system as a point of entry into the bloodstream. In addition, in a study of women undergoing gynecological surgery for removal of tumors, 46% of lymph nodes cultured yielded *Enterococcus* species, demonstrating the potential for translocation across the vaginal epithelium (38).

Evidence for enterococcal translocation across intact epithelium raises the possibility that this occurs commonly in humans, but a competent reticuloendothelial system is capable of eliminating the transient bacteremia. Perhaps administration of antibiotics leading to enterococcal overgrowth significantly increases the number of occurrences of bacterial translocation, overwhelming the reticuloendothelial system. Additionally, the reticuloendothelial system in debilitated hosts may be less efficient at clearing bacteria from the bloodstream. It is noteworthy that the administration of immunosuppressive agents, which would also limit the efficacy of the reticuloendothelial system, was found to be associated with VRE bacteremia in this study. Furthermore, many patients in this study were on chemotherapeutic regimens; in addition to suppressing the immune system, mucositis caused by such agents is likely to increase bacterial translocation, thus predisposing patients to bacteremia in multiple ways.

Regardless of the mechanism linking antibiotic exposure to VRE bacteremia, the association between the two makes hospital formulary modifications a logical method for controlling VRE. Noskin et al. reported a significant decrease in the number of VRE isolates at Northwestern Memorial Hospital after restricting the use of third generation cephalosporins and encouraging the alternative use of ampicillin / sulbactam or piperacillin (39). Also, vancomycin has been used for routine surgical prophylaxis or treatment of infections without documented β-lactam resistance, but many hospitals are employing stricter controls on its use. Measures include pharmacist review of vancomycin orders, automatic queries when prescribing vancomycin to ensure the indication is valid, and automatic stopping of vancomycin orders if criteria for continuation of therapy are not met (8). Such formulary modifications have been shown to be effective in controlling outbreaks of VRE even after barrier precautions had failed to control the outbreak. One report describes limiting vancomycin, clindamycin, and cefotaxime use, as well as encouraging the substitution of penicillins and β-lactamase

inhibitors for third generation cephalosporins during an outbreak of VRE. Through these interventions, the hospital decreased the prevalence of fecal colonization with VRE from 47% to 15 % ($p < 0.001$) (40).

Additionally, out of concern for the development of infection with VRE, the Hospital Infection Control Practices Advisory Committee (HICPAC) recommends the use of metronidazole rather than vancomycin to treat *C. difficile* colitis. Although anti-anaerobic antibiotics have been associated with VRE infection (not a significant association in this study), the risk of vancomycin use leading to VRE infection is felt to be greater.

Environmental factors

While the effect did not reach statistical significance in our study, time spent in the ICU is another risk factor for the development of VRE infections. Severity of illness in the ICU often necessitates use of multiple antibiotics (including the aforementioned antibiotics that increase risk of infection with VRE), and the need for frequent patient contact in this setting undoubtedly contributes to the spread of resistant bacteria. Inadequate hand disinfection between patient encounters is likely to play a role in transmission of VRE, and routine patient-care items may also serve as vectors for resistant bacteria. For example, the handle of a thermometer probe in one hospital was documented to have led to new VRE colonization of several patients. Identification of the implicated vector was based on genetic analysis of both the patient isolates and the isolates found on the thermometer (41). To address these potential modes of spread, HICPAC recommends dedicating patient care items such as stethoscopes, IV poles and other minor items of equipment, to the rooms of VRE patients.

In addition, alcohol-based hand cleansers may increase compliance with hand washing, thus helping to limit the spread of VRE. The institution of widely available

alcohol-based hand gels has improved hand hygiene compliance at Yale-New Haven Hospital since its introduction in August of 2001. After peaking in 2001, the number of patients with VRE bacteremia declined considerably in 2002, with only 9 such episodes documented. The addition of piperacillin / tazobactam to the formulary in September of 2000 (as an alternative to third generation cephalosporins) was the only recent change in the formulary, and the number of episodes of VRE bacteremia peaked the following year. Thus, the introduction of alcohol-based hand gels in 2001 probably played a significant role in the marked decrease in episodes of VRE bacteremia in 2002.

While it did not reach statistical significance in this study, dialysis was associated with VRE bacteremia (OR 1.92, 95% CI 0.81 – 4.54). An association between VRE and end-stage renal disease has been made previously (3, 13, 20). Vancomycin is frequently used to treat line infections caused by *Staphylococcus* species in dialysis patients. It has been suggested that renal-dosing of antibiotics (as is required for vancomycin and gentamicin – two anti-enterococcal agents) may lead to more frequent sub-therapeutic serum levels than traditional dosing (3). Whether this contributes to more frequent development of VRE in patients with end-stage renal disease is uncertain. Other factors associated with dialysis that could be associated with VRE infection could involve regular exposure to health-care equipment that is used by many different chronically ill patients. Enterococci, both those resistant to vancomycin and sensitive isolates, have been shown to persist on cotton, polyester, and plastic surfaces for more than 90 days (42). This mode of transmission among dialysis patients has not been documented, but the ability of enterococci to survive on the plastic and polyester – the same material found in dialysis machines or the furniture (beds, recliners, privacy curtains) found at dialysis centers – may be significant. Such regular exposure (a minimum of 3 times every week) to a potentially contaminated environment might increase VRE colonization if the environment is not adequately cleaned after each

patient encounter. Careful disinfection of all equipment, as well as dedicated equipment for patients who are colonized or infected with VRE could be used to limit transmission of VRE among dialysis patients.

Limitations

There are limitations to the current study. First, while this study was performed at a major university medical center, encompassing patients from a ten-year period, it is possible that the study population (including only 45 patients with clinically significant VS *E. faecium* bacteremia) was not large enough to detect a difference in outcome between VRE and VSE bacteremia. In fact, for this difference in mortality of 11.9% to be statistically significant with a power of 80%, we would have needed a total of 550 cases and controls. This is a direct consequence of our choice to include only *E. faecium* bacteremia. During the study period, there were an additional 8 patients with VRE bacteremia secondary to *E. faecalis*, and 685 patients with at least one positive blood culture for vancomycin-sensitive *E. faecalis*. However, in analyzing all blood cultures positive for *Enterococcus* species, 95% of the bacteremias in the VRE group were caused by *E. faecium*, while 85% of the VSE bacteremias were caused by *E. faecalis*. As inter-species differences would also affect outcome (confounding the effect of vancomycin-resistance on mortality), we concluded it was better to include only *E. faecium* in the test of our hypothesis. It is possible that only a multi-center study (or one at a hospital with a much higher prevalence of VRE) would generate sufficient data to detect a true difference in outcome between VR and VS *E. faecium* bacteremia, if such a difference exists.

A second limitation to the current study may be in the use of a non-standardized measure of severity of illness. The Korvick scale was first used as a measure of degree of illness in patients with *P. aeruginosa* bacteremia, and was shown to be predictive of

outcome (25). It has since been used in studies of bacteremia caused by *Enterobacter* species (26) as well as studies of enterococcal bacteremia (12, 15, 16, 24). While assessed parameters include mental status, temperature, blood pressure, and the need for mechanical ventilation, the scale does not encompass other measures of severity of disease, including co-morbid conditions (such as diabetes, coronary artery disease, oncologic diagnoses, HIV, etc.), laboratory values, and others. Our study attempted to account for co-morbid conditions by considering the need for dialysis, HIV status, requirement for surgery, and a history of organ transplant or cancer as part of the matching process. Since there was no observable difference between outcome of patients with VRE and VSE bacteremia after matching cases with controls, it is unlikely that failure to properly account for severity of illness affected our results.

Another limitation of our study may lie in our definition of clinically significant bacteremia, requiring a minimum of two positive blood cultures or one positive blood culture with another documented site of infection. While our aim was to include only those with true bacteremia in the study, surely we inadvertently excluded patients with real bacteremia that did not conform to our definition. VRE bacteremia was more likely to be considered clinically significant than VSE bacteremia (OR 1.66, 95% CI 1.02 – 2.71). While this may simply be consistent with our finding that VRE is more likely to cause persistent bacteremia (and thus more likely to satisfy our definition of clinical significance), it might also suggest that we excluded more real VSE bacteremias than VRE bacteremias. One possible way that this could occur is through rapid treatment of VSE bacteremia. As susceptibility results often take a few days to return from the laboratory (but a Gram stain can quickly reveal the presence of gram positive bacteremia), vancomycin is a common empiric treatment for gram-positive bacteremia. Administration of vancomycin might prevent follow-up cultures from being positive in the case of VSE bacteremia (and preventing an episode from meeting our criteria of clinical

significance), but not if the organism is VRE. Another possible explanation for the higher percentage of clinically significant VRE bacteremia might be that clinicians were more comfortable in dismissing VSE as a skin contaminant, and forgoing follow-up cultures if only one set was positive. However, even if these mechanisms affected our study population, it is our feeling that a single blood culture in a colonized patient may reflect contamination. True infection is may be difficult to ascertain retrospectively. Furthermore, the alternative of including all patients with a single positive culture (or those with positive rectal cultures and a single positive blood culture) could create bias by adding more patients with pseudo-bacteremia than actual bacteremia.

Finally, the limited numbers of patients in the study precluded a thorough multi-variate analysis of risk factors for VRE infection. For example, using univariate analysis, we found associations between different antibiotics and risk for VRE infection. However, many patients were on multiple antibiotics, and risk associated with vancomycin use could also be attributed to cephalosporins if the same patient were receiving multiple antibiotics. The consequence in such situations where patients received multiple antibiotics is an overstatement of the risk associated with each individual antibiotic. Similarly, while length of stay prior to development of bacteremia was associated with VRE infection, the increased likelihood of exposure to antibiotics during longer hospital stays could confound this association.

Conclusion

Vancomycin-resistance alone is not associated with increased mortality in patients with enterococcal bacteremia. However, this does not in itself obviate the importance of VRE. It has recently been demonstrated in two cases in the United States that vancomycin resistance can be transmitted from VRE to *S. aureus*, a much more serious pathogen. The potential for spread of resistance demands that we continue

efforts to control proliferation of VRE. Hospital formularies can be monitored more closely to ensure judicious use of cephalosporins and vancomycin, both of which contribute to the development of VRE. In addition, efforts to prevent transmission of VRE to dialysis, transplant, and ICU patients are indicated, as these patients are clearly at elevated risk for acquiring VRE.

Furthermore, while the difference in pathogenicity between the enterococcal species has been observed before, it remains uncharacterized and under-recognized. The exact mechanism of these differences merits further investigation. Clearly, such inter-species differences in pathogenicity demand consideration in any future studies regarding the outcome of enterococcal infections.

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